THE ALLOGENEIC BISECTION OF CARRIER-SPECIFIC ENHANCEMENT OF MONOCLONAL B-CELL RESPONSES*

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The mechanism of antigenic stimulation of bone marrow-derived precursors to antibody-forming cells (B cells) remains a complex and controversial area of research investigation. B cells have on their surface antigen receptors identical in specificity to the cell's potential secreted antibody product, and the interaction of these receptors with antigen is essential for stimulation of the B cell (1–6). Several years ago this laboratory demonstrated that this specific receptor-antigen interaction led to B-cell proliferation and subsequent antibody production only if the interaction was multivalent and the receptors exceeded a minimum threshold affinity for each antigenic determinant (7). At this point in time, however, it is not yet clear if this antigen-receptor interaction is itself sufficient for stimulation or if, for example, the B cell requires ancillary signals for triggering.

Since the classical work of Mitchison, Miller and Mitchell, Claman et al. (8–10), demonstrating a critical role for thymus-derived lymphocytes (T cells) in enhancing B cell responsiveness to certain antigens, the requisites for B-cell stimulation have been shown to be a composite of a multitude of factors differing for each antigen and, perhaps, for each different B-cell subpopulation. In addition, B-cell stimulation appears to be inhibitable by an equally complex set of factors (11–13), so that B-cell stimulation must be considered to be a result of the interplay of these two opposing processes.

At the same time a significant amount of evidence now exists for B-cell triggering in the absence of any ancillary mechanism. This evidence includes the induction of tolerance (12, 14, 15) and the generation of secondary B cells by T-dependent antigens in the absence of T cells (16), stimulation to proliferation (17) and IgM production by primary B cells by a vast array of antigens in the absence of T cells (14, 18, 19), and stimulation of even IgG production by primary B cells with selected antigens in the absence of T cells (20). However, B cells stimulated in the absence of T cells may give rise to both qualitatively and quantitatively different antibody-producing cell clones as compared to the same B cells stimulated in the presence of T cells. For example, previous studies from this laboratory have shown that secondary B cells can be stimulated by soluble hapten-carrier complexes in the absence of specific T cells (5, 7, 21). However, carrier recognition, presumably by T cells, increased both the number of responding secondary B cells (as enumerated by resultant antibody-forming cell

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clones) by 10-fold and the amount of antibody produced per clone by fourfold. In this context it may be assumed that the majority of B cells are inherently competent to be stimulated in the absence of any ancillary effects, if antigen is presented appropriately (valence, concentration, spatial array), and in the absence of extrinsic inhibitory factors. What then is the role of T cells and other ancillary mechanisms in B-cell stimulation?

Clearly antigen-specific T cells usually play a decisive role in B-cell stimulation. T cells may, for example, be essential for the appropriate presentation of antigen to the B cell either directly or through a macrophage intermediate (4, 5, 7, 9, 19). Most investigators, however, have implied a more basic role of T cells in the stimulation of B cells. In addition to facilitating antigen-receptor contact, T cells would presumably supply a second signal essential to B-cell triggering (22–29). The arguments for such a mechanism rely on: (*a*) the effectiveness of B-cell stimulation by soluble T-cell products obtained from nonspecifically-activated T cells (23–26), (*b*) the apparent dependence of T-cell–B-cell collaboration on I region syngeny (28), that is if T cells function only for antigen presentation then why is it that allogeneic carrier specific T cells do not collaborate in facilitating B-cell responses, and (*c*) the notion that T-cell independent stimulation pertains only to a subpopulation of B cells which can produce IgM antibody (30), while precursors to IgG production have an absolute T-cell requirement.

The experiments presented here address the latter two points in particular. Utilizing a stimulatory protocol with an absolute dependence on carrier-primed T cells for stimulation of primary B cells to yield antibody-forming cell clones, it is demonstrated that carrier-primed allogeneic T cells can collaborate in the stimulation of the majority of primary B cells. Stimulation of B cells by T cells allogeneic to the *Ir-1A* region of the *H-2* complex results in B-cell clones which produce small quantities of antibody and antibody that is solely of the IgM class. Since, in collaboration with syngeneic T cells, after primary stimulation the majority of B cells form clones of antibody-producing cells secreting IgG_1 antibody, it is clear that IgM antibody production is not the prerogative solely of a subpopulation of B cells. To the contrary, most B cells will yield either IgG_1 or IgM antibody-producing cell clones depending on the nature of stimulation. The implication is that the majority of B cells can be stimulated to antibody production if antigen is presented appropriately, and that the character of the resulting B-cell clone is determined by the nature of the stimulation. Triggering with most T-independent antigens or by collaboration with carrier-specific T cells allogeneic to the B cell would thus either prohibit or fail to induce the IgG_1 portion of the response.

Materials and Methods

Antigens. The preparation of *Limulus polyphemus* hemocyanin (Hy),[1] human gamma globulin (HγG), and 2,4-dinitrophenylated-hemocyanin (DNP-Hy, 10 mol of DNP per 100,000 g of Hy) have been previously described (5, 7). 3-(*p*-azophenylphosphorylcholine)-N-acetyl-L-tyrosylglycyl-

[1] *Abbreviations used in this paper*: DNP-Hy, 2,4-dinitrophenylated-hemocyanin; DNP-LYS-BAC; 2,4-dinitrophenyl-lysine-bromacetylcellulose; HγG, human gamma globulin; Hy, hemocyanin; PC, phosphorylcholine; PPC-TGG-HY, 3-(*p*-azophenylphosphorylcholine)-N-acetyl-L-tyrosylglycylglycine.

glycine Boc Hydrazide-hemocyanin (PPC-TGG-Hy), a phosphorylcholine-tripeptide-spacer-Hy conjugate, was prepared as described in another paper (31); the antigen contained 10 mol of hapten per mol Hy. Assays for the TEPC 15 idiotype were carried out as described elsewhere (32, 33).

Animals. 6–8-wk old BALB/cJ mice were obtained from Carworth Division, Becton-Dickinson & Co., Rutherford, N.J., as well as Jackson Laboratories, Bar Harbor, Maine. BALB/cAnN and A/He mice were acquired through the Institute for Cancer Research, Philadelphia, Pa. Mice from all three sources were bred in our mouse colony and used interchangeably in the studies presented. AKR/J, A/J, C_3H/J, C57/BL/10J, and B10.D2 strains of mice were purchased from Jackson Laboratories. (A/J × BALB/c)F_1 and (A/J × C57BL/10)F_1 were obtained by breeding female A/J to male BALB/c and C57BL/10, respectively, in our mouse colony. The AQR, B10.A(2R), and B10.A(4R) strains of mice used in these studies were the generous gift of Dr. W. E. Elkins who maintains a breeding colony of these mice originally obtained from the colony of Dr. Fritz Bach.

Immunizations. The various strains of mice received an intraperitoneal injection of 0.1 mg of either Hy or HγG in complete Freund's adjuvant and were used as recipients in cell transfer studies 6–12 wk later.

Cell Transfers and Fragment Cultures. The methodology for in vitro B-cell cloning and the detection of positive fragment cultures by radioimmunoassay have been previously described (7). Briefly, spleens from unimmunized mice were homogenized in Dulbecco's modified Eagle's medium using a Teflon tissue homogenizer. 4×10^6 cells from this suspension were injected intravenously into carrier-primed or normal recipients that had been irradiated at 1,300–1,600 rad 6 h earlier. Fragment cultures of the spleens of recipient mice were prepared 16 h after cell transfer. The percentage of donor cells present in recipient spleens and fragment cultures was assessed by quantitation of ^{51}Cr-labeled donor cells as previously described (34). The fragments were individually stimulated with either DNP-Hy or PPC-TGG-Hy at a hapten concentration of 10^{-6} M; culture fluids were changed every 3 days. 20 μl of culture fluids collected 9 or 12 days after stimulation were quantitatively assayed for antihapten antibody, immunoglobulin class of antibody, and the presence of the TEPC 15 idiotype in the case of anti-PC (phosphorylcholine) antibody by radioimmunoassays. These radioimmunoassays were performed by detecting immunoglobulin bound to 2,4-dinitrophenyl-lysine-bromacetylcellulose immunoadsorbent (DNP-LYS-BAC) or PC-bovine serum albumin-BAC (32) with ^{125}I-labeled antimouse Fab antibody (5, 7). Heavy chain classes were detected by the same procedure only employing ^{125}I-labeled goat antibody to mouse IgG_1 (γ_1-chain) and IgM (μ-chain) as the detecting reagent. Goat antimouse IgG_1 and goat antimouse IgM were prepared as previously described (33, 35).

Depletion of T Lymphocytes. Donor spleen cells were treated with anti-θ antiserum and complement as previously described (36). The anti-θ antiserum was prepared from the ascites fluids of AKR mice injected with C3H mouse thymocytes according to the method of Rief and Allen (37). The effectiveness of this antiserum against T cells and its lack of toxicity for B cells has been previously demonstrated (21, 36). Rabbit serum was absorbed with BALB/c spleen cells, frozen, and used as a source of complement.

Results

The Efficiency of Carrier-Specific Cooperation Across an Allogeneic Barrier. The ability of syngeneic and allogeneic spleen cells to cooperate in a humoral immune response was tested by transferring cells from nonimmune mice to heavily irradiated, Hy-primed, syngeneic, and allogeneic recipients. Fragments of these recipient spleens were cultured in vitro and stimulated with DNP-Hy. Table I summarizes the data obtained from an analysis of the culture fluids of fragments of several donor-recipient strain combinations. It can be seen that donor cells can be stimulated to yield clones of antibody-forming cells in carrier-primed recipients which are either syngeneic or allogeneic to the donor B cells at the *H-2* complex. The frequency of cells responding in the carrier-primed allogeneic environment was somewhat lower than the frequency of cells responding in the syngeneic environment although the ranges overlapped.

TABLE I
Anti-DNP Antibody-Producing Clones Obtained from Syngeneic and Allogeneic Transfers

Donor	Recipient	Hy primed	*H-2* identity*	Number of donor cells analyzed × 10^{-6}‡	Number of clones per 10^6 cells transferred§
BALB/c (d)	BALB/c (d)	+	+	40	1.95 ± 0.59
	AKR (k)	+	–	32	1.10 ± 0.48
	A/J (a)	+	–	8	0.65 ± 0.51
	CBA (k)	+	–	8	0.80 ± 0.49
AKR (k)	AKR (k)	+	+	16	1.62 ± 0.13
	BALB/c (d)	+	–	12	1.25 ± 0.46
Total‖	Hy primed	+	+	80	1.74 ± 0.31
Total	Hy primed	+	–	84	1.18 ± 0.33
Total	Nonprimed	–	+	40	0.08 ± 0.01
Total	Nonprimed	–	–	56	0.05 ± 0.01

* Donor-recipient pairs were either syngeneic, thus *H-2* identical, or different *H-2* nonidentical strains.

‡ 4 × 10^6 donor spleen cells were transferred to each recipient mouse.

§ Fragments were stimulated in vitro with DNP-Hy at 10^{-6} M DNP and clones detected by radioimmunoassay of culture fluids using ^{125}I-labeled anti-Fab.

‖ Totals represent all donor:recipient strain combinations tested including those listed and in addition: BALB/c:C_3H, C_3H:C_3H, CBA:CBA, A/J:A/J, A/He:A/He, and A/He:BALB/c. In all combinations both unprimed and Hy-primed recipients were tested.

Other investigators have implied that B cells may be nonspecifically stimulated in an allogeneic environment by the direct antigen independent interaction of allogeneically-stimulated recipient T cells with donor B cells (22, 38), or alternatively, by the allogeneic stimulation of T cells present in the donor inoculum, which in turn are capable of stimulating syngeneic donor B cells (39). Inasmuch as the donor spleen cells were transferred in limiting dilution, that is one or no hapten-specific donor B cells per recipient fragment, the effect of other donor cells, B or T, in these fragments should be minimized. This is borne out in Table II by experiments showing that, when donor cells were treated with anti-θ antiserum and complement, before transfer, both syngeneic and allogeneic responses remained essentially the same. Furthermore, the anti-DNP response of donor spleen cells was totally dependent on carrier priming of the allogeneic recipient as shown in Table I. The antigenic specificity of the carrier priming was tested using allogeneic and syngeneic recipients primed with HγG in CFA rather than Hy. Such recipients were unable to facilitate the response of donor spleen cells stimulated in vitro with DNP-Hy. Thus the stimulation of donor B cells was independent of the presence of donor T cells and apparently required specifically primed recipient T cells. These findings argue against a role for any

nonspecific, irradiation-resistant means of enhancing antigenic stimulation in this system.

In both syngeneic and allogeneic systems, the results indicate that when cells are transferred to a carrier-primed irradiated recipient, the majority of antigen-specific B cells present in fragment culture respond to antigenic stimulation. This conclusion is based on independent determinations of: (*a*) the efficiency of antigenic specific stimulation of B cells in the spleen of syngeneic recipients and (*b*) the comparative frequency of donor cells homing to the allogeneic and syngeneic spleen after cell transfer. Previous studies have determined that 70% of the BALB/c donor antigen-specific B cells, present in fragment cultures derived from syngeneic recipient spleens, are stimulated by antigen to yield antibody-forming cell clones (40, 41; Pickard and Klinman, unpublished observations). To extend this conclusion to cell transfers into allogeneic recipients, it was necessary to determine the frequency of BALB/c donor cells present in fragment cultures derived from spleens of allogeneic recipient mice. ^{51}Cr-labeled BALB/c spleen cells were transferred to carrier-primed AKR recipients. The percentage of ^{51}Cr-labeled cells present in fragment cultures after such transfers to allogeneic recipients was 5.3%, which is essentially the same as the percentage obtained after syngeneic transfer (5.1%). Thus it appears that over 50% of the primary DNP-specific BALB/c spleen cells (H-2^d) present in a carrier-primed AKR (H-2^k) spleen can be stimulated to clone formation and antibody production in fragment culture. The same conclusion presumably applies also for the efficiency of the response of AKR (H-2^k) cells transferred to BALB/c (H-2^d) recipients as well as BALB/c cells transferred to various mouse strains.

The Nature of Clonal Responses in the Carrier-Primed Allogeneic Environment. The average amount of anti-DNP specific antibody produced per day during peak synthesis by antibody-forming cell clones was quantitated by radioimmunoassay and is summarized in Table III. It can be seen that clones resulting from antigenic stimulation in splenic fragments derived from carrier-primed syngeneic recipients produced significantly more antibody than clones

TABLE II
The Effect of T-Cell Depletion on Donor Cell Responses

Donor	Hy-primed recipient	*H-2* identity	Number of donor cells analyzed × 10^{-6}	Number of clones per 10^6 spleen cells transferred*
C_3H (k)	C_3H (k)	+	8	1.50
	BALB/c (d)	–	16	1.12
C_3H (k)	C_3H (k)	+	8	1.30
after treatment with anti-θ + C′ ‡	BALB/c (b)	–	8	1.50

* Fragments were stimulated in vitro with DNP-Hy at 10^{-6} M DNP and clones detected by radioimmunoassay of culture fluids using ^{125}I-labeled anti-Fab.

‡ 4 × 10^6 spleen cells were treated with anti-θ antiserum and complement before transfer to Hy-primed recipients.

TABLE III

Quantitation of the Rate of Anti-DNP Antibody Production by Clones in Syngeneic and Allogeneic Recipients

Donor	Hy-primed recipient	*H-2* identity	Number of donor spleen cells analyzed $\times 10^{-6}$	Number clones per 10^6 spleen cells transferred*	Average nanograms anti-DNP antibody per clone per 24 h
BALB/c	BALB/c	+	32	1.90	2.40
	AKR	−	28	1.10	0.49
AKR	AKR	+	16	1.63	3.00
	BALB/c	−	12	1.25	1.50

* Fragments were stimulated in vitro with DNP-Hy at 10^{-6} M DNP. Fragment cultures were washed free of antibody on day 7. 3 days later duplicate 20-μl samples of the culture fluid were added to DNP-BAC, and antibody was quantitated by the radioimmunoassay using ^{125}I-labeled anti-Fab.

stimulated in an allogeneic environment. The fact that only small amounts of antibody were made in allogeneic recipients might have obscured the detection of such responses in less sensitive systems. The fact that less antibody was produced does not mean, however, that fewer precursor cells were stimulated, but rather that B cells stimulated in the allogeneic environment generated either fewer antibody-forming cells or cells releasing antibody at a slower rate.

Table IV presents the results of an analysis carried out to determine the heavy chain class of the monoclonal antibody synthesized by B-cell clones after stimulation in syngeneic and allogeneic carrier-primed environments. The results summarized show that B-cell responses in carrier-primed allogeneic recipients were almost totally IgM. This is in marked contrast to responses of nonimmune BALB/c spleen cells transferred to carrier-primed syngeneic recipients where only a small percentage of clones made solely IgM antibody while most clones made IgG_1 or both IgG_1 and IgM antibodies, as previously demonstrated (35).

If the majority of DNP-specific precursor cells is indeed stimulated in both allogeneic and syngeneic recipients, as argued above, then these findings imply that the same B cell, when stimulated through collaborative interactions with carrier-specific allogeneic T cells, yields less antibody and only IgM antibody. This serves as presumptive evidence that the same precursor cell under one stimulatory condition may give rise to progeny making only IgM, but when stimulated with maximal carrier-specific help in a syngeneic environment, may give rise to progeny making either IgM or IgG_1 or both. This conclusion is supported by an analysis of the response of BALB/c B cells stimulated with PPC-TGG-Hy after transfer to Hy-primed syngeneic and allogeneic recipients. The response to the PC determinant in BALB/c mice is relatively restricted, and the majority of the PC-specific B cells and the antibody product of their clonal progeny can be identified by an anti-idiotype antibody raised against the TEPC 15 myeloma protein (31–33). After stimulation in a syngeneic carrier-primed environment the majority of precursor cells, which are of the TEPC 15 idiotype,

yield clones whose antibody product is, at least in part, of the IgG or IgA class IgG_1 (32, 33). However, an analysis of five PC-specific clones derived from the transfer of BALB/c spleen cells to Hy-primed AKR and C3H recipients indicated that precursor cells of the identical TEPC 15 idiotype, and presumably of the same B-cell clone, produced only IgM antibody in a carrier-primed allogeneic environment.

The Genetic Identification of Ir-1A Region Control of Allogeneic vs. Syngeneic Type Responsiveness. Table V demonstrates that F_1 cells transferred to carrier-primed parental recipients, as well as parental cells transferred to carrier-primed F_1 recipients, yielded a significant percentage of IgG_1-producing clones. Thus, it appears that general suppressive effects of either donor cells recognizing a histoincompatible recipient environment or donor cells being recognized by irradiated histoincompatible recipient cells is not a sufficient explanation for the inability of donor cells to be stimulated to IgG_1 production in a totally histoincompatible environment.

Table V also demonstrates that clones resulting from donor and recipient strain combinations which differ in the non-*H-2* background and share only the *H-2* complex, produce IgG_1, while sharing of the non-*H-2* background does not result in IgG_1-producing clones if the donor and recipients differ in the *H-2* complex. Thus cells from the recombinant inbred congenic strain B10.D2, which shares only *H-2* region genes with BALB/c and differs from B10 only in the *H-2* gene complex, collaborate with BALB/c cells to yield IgG_1 antibody, but yield only IgM antibody in collaboration with C57BL/10 cells.

A series of experiments using a variety of donor mouse strains which differ in each case from the Hy-primed A/J recipient mice in a defined region of the *H-2*

TABLE IV
Heavy Chain Class of Anti-DNP Monoclonal Antibody Derived from Spleen Cells Transferred to Syngeneic and Allogeneic Recipients

Donor	Hy-primed recipient	*H-2* identity	Number donor spleen cells analyzed × 10^{-6}	Number clones per 10^6 spleen cells transferred	Percent clones*		
					IgM	IgG_1	IgM and IgG_1
BALB/c	BALB/c	+	32	1.73	17	54	27
	AKR	−	24	1.04	96	0	0
AKR	AKR	+	16	1.62	35	38	27
	BALB/c	−	12	1.25	86	0	7
Total all strains tested‡		+	80	1.44	21	56	23
		−	60	1.00	94	0	2

* Fragments were stimulated with DNP-Hy at 10^{-6} M DNP. The heavy chain class of ^{125}I-labeled anti-Fab positive culture fluids was analyzed using ^{125}I-labeled goat antimouse IgG_1 and IgM. The immunoglobulin class of less than 7% of the clones could not be determined by these reagents.

‡ Analysis includes, in addition to above: BALB/c transferred to CBA and A/J and C_3H transferred to C_3H and BALB/c.

complex is presented in Table VI. The underlining in Table VI emphasizes this *H-2* complex difference. It can be seen that whenever *Ir-1A* is identical in donor and recipient pairs, IgG_1-producing clones can be observed, whereas identity between other regions appears to have little consequence on the occurrence of IgG_1-producing clones. Thus, although possible contributions of regions other than *Ir-1A*, particularly *Ir-1B* and *K*, have not been excluded, it would appear that the ability of donor and recipient collaboration to yield IgG_1-producing clones is genetically closely linked to the *Ir-1A* subregion of the *H-2* complex.

Discussion

The studies presented here were carried out with a view to further elucidating the role of antigen-specific T cells in the initiation of the humoral immune response. The results contradict previous reports indicating an absolute requirement for syngeny between collaborating cells in T-cell dependent B-cell stimulation (28, 29, 38). Since responses in allogeneic recipients are small, the demonstration of efficient B-cell stimulation via collaboration with carrier-primed

TABLE V
Donor Cell Responses in Semiallogeneic Recipients and Recipients Differing in Non-H-2 Loci

Donor	Recipient	Background	Number of clones analyzed	Percent clones IgG_1 positive
CAF_1 (a/d)	A/J (a)	Shared	6	100.0*
A/J (a)	CAF_1 (a/d)	Shared	10	80.0
B10.D2 (d)	BALB/c (d)	Dissimilar	34	73.5
B10.D2 (d)	C57BL/10 (b)	Identical	24	4.2

* Percent includes clones making IgG_1 alone or IgG_1 and IgM antibody as detected using ^{125}I-labeled antimouse IgM and IgG_1 in a radioimmunoassay.

TABLE VI
H-2 Dependence of IgG_1 Anti-DNP Antibody Production

Donor cells transferred to A/J Hy-primed recipient*	*K*	*Ir-1A*	*Ir-1B*	*I-C*	*S*	*D*‡	Total clones analyzed	Percent clones IgG_1 positive
A/J	k	k	k	d	d	d	39	62
BALB/c	d	d	d	d	d	d	10	0
B10.D2	d	d	d	d	d	d	5	0
AKR	k	k	k	k	k	k	10	70
B10.A(2R)	k	k	k	d	d	b	12	66
B10.A(4R)	k	k	b	b	b	b	16	75
AQR	q	k	k	d	d	d	26	42

Underlining emphasizes the *H-2* complex genetic difference between donor cells and A/J Hy-primed recipient mice.
* In each case donor cells are transferred to Hy-primed A/J recipient mice.
‡ *H-2* complex genes as defined by Shreffler and David (42).

allogeneic T cells is partly dependent on the use of extremely sensitive techniques. The methods employed in this report combined a splenic focus technique, which enables isolation and stimulation of single specific B cells, with a sensitive radioimmunoassay for the analysis of the antibody product of that B cell's clonal progeny. These methods provide several important advantages. First, it is possible to quantitate the efficiency of any stimulatory protocol at the individual precursor cell level. Since responses in this system have been amply demonstrated to be monoclonal (7, 31–35, 40, 41, 43), it is possible to separate the effectiveness of stimulation in terms of the absolute number of precursor cells stimulated from the modulations of these stimulated cells in terms of the rate of antibody synthesis and cell proliferation. Secondly, it provides an extraordinarily sensitive means of assessing small responses by stimulated cells since each responding cell and cell clone releases its antibody into a small closed system so that minimal responses are not diluted into an entire animal or obscured by nonspecific background. Finally, these responses occur in limiting donor cell dilutions so that ancillary interactions between donor cells are minimized.

To investigate the role of T-cell–B-cell collaboration in B-cell responsiveness, it was necessary to insure the T-cell dependence of the B-cell stimulation. To this end, cells from nonimmune donors were used in cell transfers since in this system it has been previously demonstrated that the response of these cells to hapten-carrier conjugates is absolutely dependent on the carrier priming of the recipient (7). This has not proved to be the case for secondary cells, some of which can be stimulated in the total absence of specific T cells (7, 21). The present studies demonstrate that primary B cells can be antigenically stimulated by "T-dependent" antigens both in syngeneic and allogeneic recipients, and that in both instances the stimulation is dependent on the specific carrier priming of the host. It is significant that the response, even in allogeneic recipients, involved a majority of the precursor cells present. Thus, although responses are small and may be easily obscured in an intact recipient or even in some culture systems, the capacity for B-cell triggering, per se, appears to be dependent only on carrier priming and not on T cell–B cell *H-2* complex identity. Indeed, cooperation between allogeneic T cells and B cells in an antigen-triggering event, entirely dependent on carrier recognition, re-emphasizes the crucial role played by carrier recognition, presumably by T cells or T-cell products, in "antigen presentation." It has been postulated that primary cell antigen receptors may interact with antigen monovalently and thus may require accessory cells or the product of such cells for receptor interlinkage (7). Although certain antigens, either by virtue of their polymeric structure or mitogenic properties, are capable of stimulating primary B cells in the absence of T cells, soluble hapten-carrier complexes appear to require T cells for this purpose (8–10, 14, 19, 41). It would appear from the studies presented here that even carrier-specific allogeneic T cells can function in this capacity. This may imply that there is no special requirement for T-cell–B-cell recognition, in terms of a second signal, in the antigenic triggering of B cells to form antibody-producing clones.

It is significant that B cells stimulated in an allogeneic carrier-primed environment synthesize only small amounts of antibody, and this antibody is almost

entirely IgM as compared to the larger IgG_1-producing clones resulting from B-cell stimulation in a syngeneic carrier-primed environment. It is again important to emphasize that since a majority of precursor cells respond in both systems, it is very likely that a precursor cell stimulated to give IgG_1-producing progeny in the syngeneic system, produces only IgM when stimulated in the allogeneic system. This was most clearly observed when the antigen PPC-TGG-Hy was used. In this case the IgM response of donor B cells in the allogeneic recipient could be shown to be of the same idiotype as the IgG_1 response in the syngeneic recipient and therefore the product of putatively identical B cells.

The finding that collaborative interactions are maximized via carrier-specific recognition by syngeneic T cells, particularly for IgG_1 production, is consistent with previous findings that T independent stimulation usually gives rise to only IgM antibody. Recent findings by Gearhart et al. (32, 33, 44) indicate that IgG_1 production may not occur when PC-specific precursor cells are stimulated by the pneumococcal vaccine which is "T independent", but may occur when PC is presented to the same precursor cells as a hapten on a T-dependent carrier, particularly in a carrier-primed environment. Thus the mode of stimulation of a precursor cell would appear to be the most critical factor in determining the immunoglobulin class of a clone's antibody product. Although it is clear that minor B-cell subpopulations may have the capacity to yield only IgM-producing clones (45), the majority of B cells would appear to be capable of IgM, IgG_1, or IgA production depending on the nature of antigenic stimulation.

In the experimental system employed it is important to rule out any nonspecific suppressing or enhancing effects of donor or host T cells on the responding B cell. The cells were transferred in each experiment so as to obtain one or no antigen specific B cells per splenic fragment. In this case the probability of obtaining sufficient donor T cells in close proximity to the responding B cells to affect their stimulation in a majority of the fragments would be small. This argument is substantiated by experiments showing that anti-θ antiserum and complement treatment of donor spleen cells did not enhance or suppress the B-cell response. Furthermore, the responses of parental cells in carrier-primed F_1 hosts were comparable to the syngeneic response. This was also true of F_1 cells responding in a carrier-primed parental host which argues against nonspecific allogeneic effects mediated by activated host cells. Thus through the use of a relatively nonphysiological system, the stimulation of B cells via histoincompatible T cells, it has been possible to bisect the B-cell's response. That is, there appears to be a triggering signal for the stimulation of B cells by soluble hapten-protein conjugates which must be facilitated by T cells; this function is performed equally well by carrier-primed allogeneic or syngenic T cells. While stimulation to yield IgG_1 antibody-producing cells is possible for some secondary B cells even in the complete absence of specific T cells (7, 21), in order to produce IgG_1, primary B cells require not only carrier-primed T cells but carrier-primed T cells syngeneic to the stimulated B cells in the Ir region of the *H-2* gene complex.

At this time the actual mechanism by which Ia antigens may function in the immune response is not understood. The requirement for syngeneic carrier-primed T cells in the triggering of B cells to form IgG_1-synthesizing clones is in agreement with reports of other laboratories indicating that isogenicity of T and

B cells in the *Ir-1A* region is necessary to maximize B-cell responsiveness (28, 29). It is conceivable, as suggested by these investigators, that T cells collaborate with B cells partially via syngeneic Ia recognition, and that such interactions of Ia antigens, either directly with T cells or T-cell products, are essential for B-cell stimulation. If so, triggering, per se, at least for IgM production, would appear to be independent of such recognition.

An alternative explanation of the need for *Ir-1A* syngeny is the potential inhibition of B-cell responses in the presence of allogeneic T cells reactive to the *H-2* complex antigens of the responding B cells. This interpretation would be consistent with recent observations by von Boehmer (personal communication) and Katz and Wilson (46), as well as the response of nonresponder B cells noted in allophenic mice (47). In each case, cell populations, presumably deficient in T cells responsive to the B-cell alloantigens, appear to have excellent T-cell–B-cell collaboration in the absence of histocompatibility antigen syngeny. Such an interpretation may, however, be an oversimplification of experimental findings since: (*a*) the responding B cells in allogeneic fragment cultures are only suppressed in their capacity to make IgG_1, while clone formation and IgM synthesis occur with great efficiency, (*b*) responses of F_1 cells in parental recipients are indistinguishable from syngeneic responses, (*c*) incompatibility in regions other than Ir has no effect, and (*d*) secondary B cells respond with IgG_1 production in noncarrier primed allogeneic recipients (S. Pierce, J. Press, and N. Klinman, unpublished observations). Thus, if suppression via histoincompatibility is responsible for the inability of nonisogenic T and B cells to collaborate, such suppression is likely to be at the level of Ia antigens and their role in stimulation.

Such a role for Ia antigens in enabling B-cell stimulation to be maximized in terms of IgG_1 production is implied by recent studies which show that the small percentage of B cells which lack demonstrable Ia determinants generally produce only IgM antibody upon stimulation (45). In this report, Dr. Press has suggested that Ia antigens on B cells may be an intrinsic element in the maximal stimulation of mature B cells, and that T cells may only serve as an antigen presentation mechanism. If stimulation requires carrier-specific T cells, such T cells, if allogeneic, may interfere with the normal Ia function and thus lead to an abortive IgM type stimulation. Such a theory would be consistent with the findings that: (*a*) certain antigens (DNP-Ficoll) can stimulate primary B cells to IgG production in the absence of T cells (20) and (*b*) some secondary cells can be stimulated by soluble antigens to IgG_1 production in the absence of T cells (7, 21).

Regardless of the mechanistic differences inherent in B-cell stimulation with allogeneic vs. syngeneic T cells, the ability to obtain at least abortive responses with allogeneic T cells should permit a more detailed analysis of the role of T cells in the stimulation of B-cell subpopulations as well as the role of T cells in the generation of memory and tolerance. In addition, these findings demand that extremely careful analysis for small amounts of IgM antibody must be carried out before investigators can imply an absence of triggering.

Summary

The ability of T cells to enhance the response of syngeneic and allogeneic B cells to thymus-dependent hapten-carrier conjugates was analyzed. This analy-

sis was carried out on individual primary B cells in splenic fragment cultures derived from irradiated reconstituted mice. This system has several advantages: (*a*) the response of the B cells is entirely dependent on carrier priming of the irradiated recipient; (*b*) this B-cell response can be quantitated in terms of the number of responding cells; and (*c*) very small B-cell responses can be readily detected and analyzed. The results indicate that the majority of hapten-specific B cells were stimulated in allogeneic and syngeneic recipients only if these recipients were previously carrier primed. The number of B cells responding in carrier-primed allogeneic recipients was 60–70% of that in syngeneic carrier-primed recipients. The antibody-forming cell clones resulting from B cells stimulated in the allogeneic environment produced small amounts of antibody and antibody solely of the IgM immunoglobulin class, while the larger responses in syngeneic recipients were predominantly IgG_1 or IgM plus IgG_1. The capacity of collaborative interactions between carrier-primed T cells and primary B cells to yield IgG_1 antibody-producing clones was shown to be dependent on syngeny between these cells in the *H-2* gene complex. It is concluded that: (*a*) B cells can be triggered by T-dependent antigens to clone formation through collaboration with T cells which differ at the *H-2* complex as long as these T cells recognize the antigen; (*b*) the immunoglobulin class produced by the progeny of stimulated B cells generally depends on the nature of the stimulatory event rather than the nature of the B cell itself; and (*c*) stimulation to IgG_1 production is dependent on syngeny between the collaborating T and B cells probably within the *Ir-1A* region. The role of the Ia antigens in the formation of IgG_1-producing clones is not yet clear; Ia identity could permit IgG_1 production or, conversely, nonidentity of Ia could induce allogeneic interactions which prohibit IgG_1 production.

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